The Examiner maintains that the phase "chemotherapeutic stress stimulus" has no support in the specification. As stated in MPEP § 2163.02, "[t]he subject matter of the claim need not be described literally (i.e. using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement." While support for a claim must be found in the originally filed disclosure, such support may be in the form of express, implicit or inherent support. *See* MPEP § 2163.05. Support for "chemotherapeutic stress stimulus" is implicit or inherent in the originally filed disclosure. The present invention is directed to, *inter alia*, methods for identifying compounds in which a cell is exposed to a chemotherapeutic stress stimulus to induce apoptosis (see, e.g., the specification at page 1, line 10, page 4, line 13, page 11, lines 17-30 and page 14, lines 19-22). The specification as filed provides express support for a stress stimulus which can be a "chemotherapeutic agent".

The Examiner also maintained a new matter rejection for "chemotherapeutic stress stimulus" as allegedly not having support in the specification. Applicants respectfully disagree for the reasons of record and as set forth above. Applicants previously submitted a copy of the following reference: Dennis A. Casciato and Barry B. Lowitz, *Manual of Clinical Oncology*, Chapter 4 entitled "*Cancer Chemotherapeutic Agents*" pp. 33-75 (1995) (**Reference CJ**). Casciato and Lowitz enumerated and catagorized numerous chemotherapeutic agents which were well known in the art before the effective filing date of the instant application. To advance prosecution, without conceding the correctness of this rejection, Applicants have now amended the claims to recite "chemotherapeutic agent" in all pending claims.

In view of the foregoing amendments and remarks, reconsideration and withdrawal of this rejection is respectfully requested.

THE REJECTION UNDER 35 U.S.C. § 103(a)

Claims 1-3, 5, 7 and 9-13 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lowe, S.W., *et al.*, 1993, *Cell* 74:957-67 ("*Lowe*") in view of Jarvis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:73-77 ("*Jarvis*"), Cifone, M. G. et al., 1995, *EMBO J.* 14: 5859-68 ("*Cifone*") and Schuchman et al., 1998, U.S. Pat. No. 5,773,278, entitled "Acid Shingomyelinase Gene," issued June 30, 1998, filed May 3, 1991 ("*Schuchman*") or Horinouchi, K., et al., 1995, *Nature Genetics* 10: 288-93 ("*Horinouchi*") or Otterbach, B., et al., 1995, *Cell* 81:1053-61 ("*Otterbach*").

In response, Applicants respectfully traverse the rejection. None of the cited references, either alone or in combination, teach or suggest the invention as defined in the claims now pending for the reasons of record and as set forth below. The objective standard for obviousness under 35 U.S.C. § 103 was made of record in Applicants' June 12, 2002 reply.

THE PRESENT INVENTION

Applicants' invention is directed, *inter alia*, to methods for identifying compounds which increase a cell's sensitivity to acid sphingomyelinase-related apoptosis. Acid sphingomyelinase cells are exposed to a chemotherapeutic stress stimulus, in the presence or absence of a test compound, to determine the effect of a test compound on apoptosis. In one embodiment, compounds can be identified which mimic acid sphingomyelinase or act downstream of acid sphigomyelinase in apoptotic pathways and increase a neoplastic cell's sensitivity to apoptosis, thus improving the clinical effects of anti-cancer therapy (*see* the specification, at page 4, lines 13-22). In another embodiment, compounds can be identified which decrease a cell's sensitivity to acid sphigomyelinase-related apoptois, thereby minimizing the effects of stress-induced apoptosis (*see* the specification, at page 5, lines 13-20.

THE REFERENCES

As a preliminary matter, Applicants respectfully submit that, contrary to the Examiner's position, Applicants did not attack the references individually in the June 12, 20002 reply. Rather, Applicants summarized the teachings of each reference, then argued it was improper to combine them. Applicants also pointed out that, even if properly combined, the cited references do not teach or suggest all claim limitations.

Briefly, *Lowe* does not teach methods to identify any compounds, must less methods to identify compounds that increase or decrease a cell's sensitivity to acid sphingomyelinase-related apoptosis.

While the present invention and *Lowe* both involve exposing a cell to chemotherapeutic agents, the critical difference between the present invention and *Lowe* is that *Lowe* fails to teach or suggest identifying test compounds which modulate a cell's sensitivity to chemotherapeutic agents. Contrary to the Examiner's interpretation, *Lowe*

demonstrated that p53, not any "test compounds" modulates a cell's sensitivity to chemotherapeutic agent stress stimuli. Note the mechanisms of action of the chemotherapeutic agents used in Lowe. As stated in *Lowe*, at page 959, col. 2, lines 4-7, 5-fluorouracil is an anti-metabolite, etoposide is a topoisomerase II inhibitor, and adriamycin is an intercalating agent. None of these compounds directly affects p53 or its pathway. By contrast, in the present invention, test compounds are selected based on their ability to affect the acid sphigomyelinase pathway.

Jarvis teaches that exogenously added neutral sphingomyelinase from bacteria (*Staphylococcus aureus*) and synthetic ceramides selectively induce apoptosis. Importantly, *Jarvis* does not teach the role of acid sphingomyelinase in the sphingomyelin pathway examined therein. Only the role of neutral spingomyelinase is discussed. *Jarvis* at 73.

At the time of the present invention, acid sphingomyelinase was generally considered to be a lysosomal enzyme and ceramide produced by acid sphigomyelinase was thought not to be involved in apoptosis. See, e.g. Jaffrezou, J.-P. *et al.*, 1996, EMBO J. 15:2417-2424 ("Jaffrezou"; **Reference CK**) at page 2420 (where exposure to daunorubicin caused an increase in neutral sphinogmyelinase activity but not acid sphingomyelinase activity). Further, there were conflicting results on whether acid sphingomyelinase was involved in apoptosis. For a review of the field, *see* Segui, B. et al., 2000, FASEB J. 14:36-47 at page 37, col. 1, lines 3-32 and page 44, col.1, line 35 *et seq* ("Segui"; **Reference CL**). Thus, *Jarvis*' use of neutral sphingomyelinase provides no teaching or suggestion for the use of acidic sphigomyelinase.

While *Cifone* discusses the potential role of acidic sphingomyelinase in apoptosis, the role of acidic sphingomyelinase in apoptosis was controversial and it was generally accepted that acid sphingomyelinase was not involved in apoptosis. The fact that the present invention proceeded contrary to accepted wisdom is evidence of nonobviousness. *See* MPEP § 2145(X)(D)(3). The totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. *In re Hedges*, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986). The Jaffrezou and Segui references cited above provide such evidence.

Schuchman discloses the full length human acid sphingomyelinase nucleotide and protein sequences and methods of producing transgenic mice that may serve as models for Niemann-Pick Disease in humans. *Horinouchi* teaches acid sphingomyelinase ("ASM")

deficient mice as a model for Niemann-Pick disease types A and B. *Otterbach* also teaches how to produce transgenic ASM deficient mice. However, compound identification methods are not taught in any of these disclosures. Specifically, *Schuchman, Horinouchi* and *Otterbach* do not teach or suggest a method for identifying compounds that modulate acid sphingomyelinase-related apoptosis by exposing certain cells to a chemotherapeutic agent stress stimulus, as recited in all amended claims.

First, there is no suggestion or motivation to modify the references. "The test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. Where the teachings of two or more prior art references conflict, the examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991)". *See* MPEP § 2143.01. In the instant case, the teachings of the prior art references with respect to the role of acid sphingomyelinase in apoptosis are in conflict.

Second, there is not a reasonable expectation of success. "Obviousness does not require absolute predictability, however, at least some degree of predictability is required. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976)". *See* MPEP § 2143.02. As discussed above, the state of the art was not predictable at the time the invention was made. Thus, one of skill in the art would not have a reasonable expectation of success.

Third, not all the claims limitations are taught or suggested by the cited references. "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). *See* MPEP § 2143.03. As discussed above, none of the references teaches or suggests a method for identifying compounds that modulate acid sphingomyelinase-related apoptosis by exposing certain cells to a chemotherapeutic agent stress stimulus. Specifically, the use of test compounds to identify a compound that modulates an acid sphingomyelinase apoptotic pathway is not taught or suggested by any of the cited references.

Accordingly, the present invention is not obvious in view of the cited references. In view of the foregoing, Applicants submit that this rejection has been obviated or overcome. Reconsideration and withdrawal are respectfully requested.

CONCLUSION

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. An early allowance is earnestly sought. No fee is believed to be due in connection with the amendments made in this Reply. If any fee is due, however, please charge the required fee to Pennie & Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

Date December 9, 2002

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Enclosures

EXHIBIT A

Attorney Docket No. 6923-106

U.S. Application No. 09/928,872

Marked-Up Version to Show Changes Made in Claims

December 9, 2002

1. (Amended) A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting an acid sphingomyelinase-deficient cell with a test compound;

(b) exposing the cell to a chemotherapeutic [stress stimulus] agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity;

(c) exposing an acid sphingomyelinase-deficient cell, in the absence of the test compound, to the chemotherapeutic [stress stimulus] agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(d) monitoring the exposed cells of steps (b) and (c) for the presence of an apoptotic morphology,

such that if the cell from step (b) exhibits a more severe apoptotic morphology, than that of the cell from step (c) the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

2. (Amended) A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting an acid sphingomyelinase-deficient cell with a test compound;

(b) exposing the cell to a chemotherapeutic [stress stimulus] agent;

(c) exposing an acid sphingomyelinase-deficient cell, in the absence of the test compound, to the chemotherapeutic [stress stimulus] agent; and

(d) comparing the levels of sphingomyelin and ceramide present in the exposed cell of step (b) to the levels present in the exposed cell of step (c),

such that if the level of sphingomyelin in the cell of step (b) is less than that of the cell of step (c), or the level of ceramide in the cell of step (b) is greater than that of the cell in step (c), the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

5. (Amended) A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting a cell exhibiting acid sphingomyelinase activity with a test compound;

(b) exposing the cell to a chemotherapeutic [stress stimulus] agent;

(c) exposing a cell exhibiting acid sphingomyelinase activity to the chemotherapeutic [stress stimulus] agent, in the absence of the test compound; and

(d) comparing the levels of sphingomyelin and ceramide present in the exposed cell of step (b) to the levels present in the exposed cell of step (c),

such that if the level of sphingomyelin in the cell of step (b) is greater than that of the cell of step (c), or the level of ceramide in the cell of step (b) is less than that of the cell in step (c), the test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

10. (Amended) A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) exposing acid sphingomyelinase-deficient cells, wherein the cells are part of cell lines or a genetically engineered nonhuman animal deficient for the acid sphingomyelinase gene, in the presence or the absence of a test compound, to a chemotherapeutic [stress stimulus] agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(b) monitoring the exposed cells of step (a) for the presence of an apoptotic morphology, such that if the cells treated with the test compound exhibit a more severe apoptotic morphology than that of the cells not treated with the test compound, the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

11. (Amended) A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) exposing acid sphingomyelinase-deficient cells, wherein the cells are part of cell lines or a genetically engineered nonhuman animal deficient for the acid sphingomyelinase gene, in the presence or the absence of a test compound, to a chemotherapeutic [stress stimulus] agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with test compound to cells untreated with the test compound, such that if the level of sphingomyelin in the cells treated with the test compound is less than that of cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is greater than in cells not treated with the test compound, the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

12. (Amended) A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising,

(a) exposing transgenic cells, comprised of cells deficient in endogenous acid sphingomyelinase gene activity that contain a functional human acid sphingomyelinase gene capable of expressing functional human acid sphingomyelinase, to a chemotherapeutic [stress stimulus] agent in the presence or absence of a test compound; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with test compound to cells not treated with the test compound, such that if the level of sphingomyelin in cells treated with the test compound is greater than in cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is less than that of cells not treated with the test compound, the test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

13. (Amended) A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising,

(a) exposing cells, wherein the cells are genetically engineered cells that exhibit a greater level of acid sphingomyelinase activity than non-genetically engineered cells of the same type, to a chemotherapeutic [stress stimulus] agent in the presence or absence of a test compound; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with the test compound to cells not treated with the test compound, such that if the level of sphingomyelin in cells treated with the test compound is greater than in cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is less than that of cells not treated with test compound, the test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

EXHIBIT B

Attorney Docket No. 6923-106

U.S. Application No. 09/928,872

All Pending Claims After Entry of Instant Amendment

December 9, 2002

1. A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting an acid sphingomyelinase-deficient cell with a test compound;

(b) exposing the cell to a chemotherapeutic agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity;

(c) exposing an acid sphingomyelinase-deficient cell, in the absence of the test compound, to the chemotherapeutic agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(d) monitoring the exposed cells of steps (b) and (c) for the presence of an apoptotic morphology,

such that if the cell from step (b) exhibits a more severe apoptotic morphology, than that of the cell from step (c) the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

2. A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting an acid sphingomyelinase-deficient cell with a test compound;

(b) exposing the cell to a chemotherapeutic agent;

(c) exposing an acid sphingomyelinase-deficient cell, in the absence of the test compound, to the chemotherapeutic agent; and

(d) comparing the levels of sphingomyelin and ceramide present in the exposed cell of step (b) to the levels present in the exposed cell of step (c),

such that if the level of sphingomyelin in the cell of step (b) is less than that of the cell of step (c), or the level of ceramide in the cell of step (b) is greater than that of the cell in step (c), the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

3. The method of Claim 1 or 2 wherein the acid sphingomyelinase-deficient cell is part of a genetically engineered nonhuman animal deficient for the acid sphingomyelinase gene.

5. A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) contacting a cell exhibiting acid sphingomyelinase activity with a test compound;

(b) exposing the cell to a chemotherapeutic agent;

(c) exposing a cell exhibiting acid sphingomyelinase activity to the chemotherapeutic agent, in the absence of the test compound; and

(d) comparing the levels of sphingomyelin and ceramide present in the exposed cell of step (b) to the levels present in the exposed cell of step (c),

such that if the level of sphingomyelin in the cell of step (b) is greater than that of the cell of step (c), or the level of ceramide in the cell of step (b) is less than that of the cell in step (c), the test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

7. The method of Claim 12 wherein the cell is part of a genetically engineered nonhuman animal deficient in endogenous acid sphingomyelinase gene activity and containing integrated in its cells a functional human acid sphingomyelinase transgene capable of expressing functional human acid sphingomyelinase.

9. The method of Claim 1 or 10 wherein the apoptotic morphology comprises cellular condensation, nuclear condensation or zeiosis.

10. A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) exposing acid sphingomyelinase-deficient cells, wherein the cells are part of cell lines or a genetically engineered nonhuman animal deficient for the acid sphingomyelinase gene, in the presence or the absence of a test compound, to a chemotherapeutic agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(b) monitoring the exposed cells of step (a) for the presence of an apoptotic morphology, such that if the cells treated with the test compound exhibit a more severe apoptotic morphology than that of the cells not treated with the test compound, the test compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

11. A method for identifying a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising:

(a) exposing acid sphingomyelinase-deficient cells, wherein the cells are part of cell lines or a genetically engineered nonhuman animal deficient for the acid sphingomyelinase gene, in the presence or the absence of a test compound, to a chemotherapeutic agent for a time sufficient to induce apoptosis in a cell exhibiting normal acid sphingomyelinase activity; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with test compound to cells untreated with the test compound, such that if the level of sphingomyelin in the cells treated with the test compound is less than that of cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is greater than in cells not treated with the test compound, the test

compound represents a compound which increases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

12. A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising,

(a) exposing transgenic cells, comprised of cells deficient in endogenous acid sphingomyelinase gene activity that contain a functional human acid sphingomyelinase gene capable of expressing functional human acid sphingomyelinase, to a chemotherapeutic agent in the presence or absence of a test compound; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with test compound to cells not treated with the test compound, such that if the level of sphingomyelin in cells treated with the test compound is greater than in cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is less than that of cells not treated with the test compound, the test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.

13. A method for identifying a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis, comprising,

(a) exposing cells, wherein the cells are genetically engineered cells that exhibit a greater level of acid sphingomyelinase activity than non-genetically engineered cells of the same type, to a chemotherapeutic agent in the presence or absence of a test compound; and

(b) comparing the levels of sphingomyelin and ceramide present in cells treated with the test compound to cells not treated with the test compound, such that if the level of sphingomyelin in cells treated with the test compound is greater than in cells not treated with the test compound, or the level of ceramide in cells treated with the test compound is less than that of cells not treated with test compound, the

test compound represents a compound which decreases a cell's sensitivity to acid sphingomyelinase-related apoptosis.